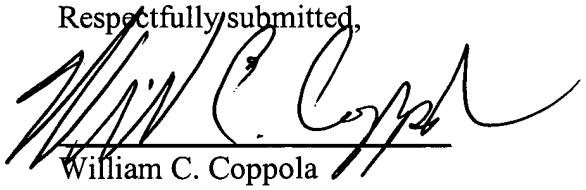
*Fees*

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account no. 18-1982 for any underpayment, or to credit any overpayments.

CONCLUSION

Applicants respectfully submit that the Claims as amended are believed to be in condition for allowance. Thus, early and favorable action on the claims is earnestly solicited.

Respectfully submitted,



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**Version With Markings To Show Changes Made**

Added material is underlined and italicized, and removed material is within brackets.

IN THE SPECIFICATION

Page 23, second paragraph:

Other signals are involved in mitochondrial targeting (for example the N-terminal part of rat ornithine transcarbamylase (OTC) allows the targeting of mitochondria) or in homing onto the endoplasmic reticulum. Finally, some signals allow nuclear retention or retention at the level of the endoplasmic reticulum (such as the sequence KDEL *(SEQ ID NO: 23)*).

Page 32, second full paragraph:

Figure 2: Analysis, on a 15% polyacrylamide gel, of the oligonucleotide-peptide (Pso-$GA_{19}$-NLS *(SEQ ID NO: 4 and SEQ ID NO:20)* chimera by proteolytic action of trypsin.

1 = oligo Pso-$GA_{19}$-SH *(SEQ ID NO:4)*

2 = oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*

3 = oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)* after digestion with trypsin.

Page 32, fourth paragraph:

Figure 5: analysis on a 15% polyacrylamide gel of the formation of triple helices between the plasmid pXL2813 and the oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*.

The oligonucleotide pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* and the plasmid are

mixed in a buffer containing 100 mM $MgCl_2$. The molar excess of oligonucleotide relative to the plasmid varies from 0 to 200. The mixture is photoactivated, after leaving overnight at 37EC, and then digested with two restriction enzymes which cut the plasmid on either side of the region of formation of the triple helices.

1 = no oligonucleotide

2 = molar excess of oligonucleotide relative to the plasmid of 15

3 = molar excess of oligonucleotide relative to the

Page 33, third paragraph:

Figure 7: Characterization of the peptide part of the oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)* by interaction with importin 60-GST (analysis on a 15% polyacrylamide gel).

1 = oligo Pso-$GA_{19}$ (1µg) *(SEQ ID NO:4)*

2 = oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera (1µg) *(SEQ ID NO:4 and SEQ ID NO:20)*

3 = supernatant recovered after incubation of glutathione-beads coated with importins 60 and the oligonucleotide Pso-$GA_{19}$ *(SEQ ID NO:4)*, and separation of the pellet of beads (containing the components which interact with the importins) from the supernatant

4 = pellet recovered after incubation of glutathione-

Page 34, first and second paragraphs:

beads coated with importins 60 and the oligonucleotide Pso-$GA_{19}$ *(SEQ ID NO:4)*, and separation of the pellet of beads (containing the components which interact with the importins)

from the supernatant

5 = supernatant recovered after incubation of the glutathione-beads coated with importins 60 and the oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*, and separation of the pellet of beads (containing the components which react with the importins) from the supernatant

6 = pellet recovered after incubation of the glutathione-beads coated with importins 60 and the oligonucleotide-peptide Pso-$GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*, and separation of the pellet of beads (containing the components which interact with the importins) from the supernatant.

Figure 8: Analysis on a 15% polyacrylamide gel of the oligonucleotide-peptide ($GA_{19}$-NLS) *(SEQ ID NO:4 and SEQ ID NO:20)* chimera by the proteolytic action of trypsin.

1 = oligo $GA_{19}$-SH (200 ng) *(SEQ ID NO:4)*

2 = oligonucleotide-peptide $GA_{19}$-NLS chimera (1µg) *(SEQ ID NO:4 and SEQ ID NO:20)* before purification by high-performance liquid chromatography

3 = oligonucleotide-peptide $GA_{19}$-NLS chimera (1µg) *(SEQ ID NO:4 and SEQ ID NO:20)* after purification

4 = oligonucleotide-peptide $GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)* after

Page 35: remove the first and second paragraphs and insert therefor:

Figure 9: Graphical representation of the kinetics of formation of the triple helices (% of triple helix sites occupied as a function of time) between the plasmid pXL2813 and the chimera $GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)*.

Figure 10: Characterization of the peptide part of the oligonucleotide-peptide $GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)* by interaction with the importin 60-GST.

1 = oligonucleotide-peptide $GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*,

2 = oligo $GA_{19}$ *(SEQ ID NO:4)*,

3 = supernatant recovered after incubation of the glutathione-beads coated with importins 60 and the oligonucleotide-peptide $GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*, and separation of the pellet of beads (containing the components which interact with the importins) from the supernatant,

4 = pellet recovered after incubation of the glutathione-beads coated with importins 60 and the oligonucleotide-peptide $GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*, and separation of the pellet of beads (containing the components which interact with the importins) from the supernatant,

5 = supernatant recovered after incubation of the glutathione-beads coated with importins 60 and the oligonucleotide-peptide [$GA_{19}$] *$GA_{19}$-NLS chimera (SEQ ID NO:4 and SEQ ID NO:20)* and separation of the pellet of beads (containing the components which interact with the importins) from the supernatant,

6 = pellet recovered after incubation of the

Page 35, first paragraph:

glutathione-beads coated with importins 60 and the oligonucleotide $GA_{19}$ *(SEQ ID NO:4)*, and separation of the pellet of beads (containing the components which interact with the importins) from the supernatant.

Page 37, second paragraph:

The oligonucleotides noted $GA_{19}$-SH *(SEQ ID NO:4)* or pim-SH *(SEQ ID NO:15)* have the same sequences as $GA_{19}$ *(SEQ ID NO:4)* and pim *(SEQ ID NO:15)*, respectively, and a thiol group at the 5' end, with a spacer of six carbon between the thiol and the phosphate of the 5' end. The oligonucleotides noted Pso-$GA_{19}$-SH (SEQ ID NO:4) have a thiol group at the 3' (SH) end,

and in addition a psoralen at the 5' (Pso) end, with a spacer of six carbons between the psoralen and the phosphate of the 5' end. The oligonucleotides noted Pso-$GA_{19}$ *(SEQ ID NO:4)* do not have a thiol group.

Page 37, Table I:

| name of the oligonucleotide | Modification of the 3' end | modification of the 5' end |
|---|---|---|
| $GA_{19}$ *(SEQ ID NO:4)* | none | None |
| Pim *(SEQ ID NO:15)* | none | None |
| $GA_{19}$-SH *(SEQ ID NO:4)* | none | thiol group |
| pim-SH *(SEQ ID NO:15)* | none | thiol group |
| Psi-$GA_{19}$ *(SEQ ID NO:4)* | none | Psoralen |
| Pso-$GA_{19}$-SH *(SEQ ID NO:4)* | Thiol group | Psoralen |

Page 38, third paragraph:

These peptides also carry a spacer of four amino acids at the N-terminal end: KGAG *(SEQ ID NO:22)*. The N-terminal lysine is chemically modified: it contains a maleimide group and the $\epsilon$ carbon and a protecting group 9-fluorenylmethyloxycarbonyl (Fmoc) on the amine of the $\alpha$ carbon. This Fmoc group absorbs at 260 nm, which makes it possible to monitor the peptide by reversed-phase high-performance liquid chromatography. The C-terminal group is

also protected ($CONH_2$ group), the protection being added at the end of peptide synthesis.

Page 39, Table II:

| name of the peptide | sequence and modifications |
|---|---|
| Maleimide-NLS *(SEQ ID NO:20)* | Maleimide-KGAGPKKKRKV-$CONH_2$ ] Fmoc |
| Maleimide-NLSmutated *(SEQ ID NO:21)* | Maleimide-KGAGPKNKRKV-$CONH_2$ ] Fmoc |

Page 50, first and second paragraphs:

This example illustrates the possibility of coupling the oligonucleotide Pso-$GA_{19}$-SH *(SEQ ID NO:4)* to the peptide maleimide-NLS *(SEQ ID NO:20)*.

The oligonucleotide Pso-$GA_{19}$-SH, of sequence 5'-AAGGAGAGGAGGGAGGGAA-3', SEQ ID No. 4) with a thiol group at the 3' end, was coupled to the peptide NLS *(SEQ ID NO:20)* which carries a maleimide group at its N-terminal end according to the method described above in the "Materials and Methods" part under the section "coupling of the oligonucleotides and the peptides".

Page 50, fifth paragraph:

The oligonucleotide-peptide (Pso-$GA_{19}$-NLS) chimera *(SEQ ID NO:4 and SEQ ID NO:20)* was analysed by polyacrylamide gel electrophoresis after proteolytic action of trypsin which makes it possible to reveal the presence of the peptide part of the chimera, after migration on a denaturing polyacrylamide gel and silver staining of

Page 51, second, third, fourth and fifth paragraphs:

The chimera Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* exhibits a retarded electrophoretic migration compared with the oligonucleotide Pso-$GA_{19}$-SH *(SEQ ID NO:4)*, and the product of proteolytic digestion is visualized at an intermediate level between the levels of migration of Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* and of Pso-$GA_{19}$-SH *(SEQ ID NO:4)*, as shown in Figure 2.

The chimera Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* therefore contains a peptide part which is accessible to trypsin. These results clearly show that the coupling between the oligonucleotide and the peptide occurred, and the coupling yield is high.

**Example 2**

This example illustrates the formation of triple helices between the plasmid pXL2813 and the chimera Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* which is modified by a photoactivable alkylating agent. This example also indicates the proportion of plasmids modified according to the molar excess of oligonucleotides relative to the plasmid.

The plasmid pXL2813, represented in Figure 3, comprises the homopurine sequence complementary to $GA_{19}$ *(SEQ ID NO:4)* which is capable of forming a triple helix with the

Page 52, first paragraph:

oligonucleotides $GA_{19}$ *(SEQ ID NO:4)*, Pso-$GA_{19}$ *(SEQ ID NO:4)*, Pso-$GA_{19}$-SH *(SEQ ID NO:4)* or Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)*. Divalent cations such as $Mg^{2+}$ stabilize these triple helices. The oligonucleotide Pso-$GA_{19}$-NLS and the plasmid are mixed in a buffer containing 100 mM of $MgCl_2$. The molar excess of oligonucleotide relative to the plasmid varies from 0 to 200.

Page 53, first, second and third full paragraphs:

The results are indicated in Figure 5. For a molar excess of oligonucleotide relative to the

plasmid greater than 50, all the plasmids are modified and are combined with an oligonucleotide Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)*. Without photoactivation, the retardation of the digestion fragment is lost in a denaturing gel.

It thus appears that the triple helices formed with the oligonucleotides Pso-$GA_{19}$-SH *(SEQ ID NO:4)* or Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* are therefore indeed covalently bound to the double helix after photoactivation.

Moreover, by digesting the remainder of the plasmid backbone and by analysing as above, it is possible to check that the photoactivation does not result in a nonspecific covalent binding of the oligonucleotide Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* outside the region containing the sequence capable of forming a triple helix.

Page 54, second full paragraph:

The expression of β-galactosidase by the plasmids pXL2813 which are nonmodified or which are combined with an oligonucleotide Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* was thus compared by transfection of NIH3T3 cells.

Page 57, second, third and four paragraphs:

This example illustrates the characterization of the peptide part of the Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* conjugates, that is to say the verification of the targeting properties of the NLS peptide *(SEQ ID NO:20)* combined with the constructs according to the invention.

The peptide sequence used, the NLS signal of the SV40 T antigen *(SEQ ID NO:20)*, is recognized by receptors of the α-karyopherin family. The murine equivalent, called importin 60, fused with a glutothione S-transferase group, was used to characterize the oligonucleotide-peptide conjugates. It was performed according to the method described in the "Materials and

Methods" part under the section "Interaction with the importins".

The interactions between the glutathion-beads coated with importins 60 and the oligonucleotides $GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* or Pso-$GA_{19}$-NLS *(SEQ ID NO:4 or SEQ ID NO:20)* were studied. After incubating for 30 minutes at room temperature, the pellet of beads (containing the components which interact with the importins) is separated from the supernatant.

The result of this characterization is reported in Figure 7. This figure indicates that the oligonucleotide Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* is combined with the

Page 58, the second and third paragraphs:

This clearly shows the capacity of the oligonucleotide Pso-$GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* to interact with the α-importin. The peptide part of the oligonucleotide-peptide chimeras is therefore indeed recognized by its receptor, which means that the peptide effectively fulfils its role of targeting signal.

**Example 6**

This example illustrates the possibility of coupling the oligonucleotide $GA_{19}$-SH *(SEQ ID NO:4)* to the peptide maleimide-NLS. Unlike Example 1, the chimera is not modified by a photoactivable alkylating agent.

The oligonucleotide $GA_{19}$-SH, of sequence 5'-AAGGAGAGGAGGGAGGGAA-3' (SEQ ID No. 4), with a thiol group at the 5' end, was coupled to the peptide maleimide-NLS *(SEQ ID NO:20)* which possesses a maleimide group at its N-terminal end under the same conditions as for the oligonucleotide Pso-$GA_{19}$-SH *(SEQ ID NO:4)* (see Example 1).

Page 59, second, third and fourth paragraphs:

The result is represented in Figure 8. It is comparable to that obtained with the oligonucleotide Pso-$GA_{19}$-SH.

**Example 7**

This example illustrates the possibility of forming triple helices between the plasmid pXL2813 and the GA-$_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)* in the absence of alkylating agent.

A kinetics of formation of triple helices between the plasmid pXK2813 and the GA-$_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*, obtained as described in Example 5, was carried out and studied according to the technique described in the "Materials and Methods" part, under the section "Formation of triple helices with the chimeras".

Page 60, paragraphs 1-5:

of the peptide part of the $GA_{19}$-NLS chimera *(SEQ ID NO:4 and SEQ ID NO:20)*.

The peptide sequence used, the NLS signal of the SV40 T antigen *(SEQ ID NO:20)*, is recognized by receptors of the α-karyopherin family, as already mentioned in Example 4. The murine equivalent, called importin 60, fused with a glutathione S-transferase group, was used to characterize the oligonucleotide-peptide conjugates. It was performed as described in "Materials and Methods", under the section "Interactions with the importins".

The interactions between the glutathione-beads coated with importins 60 and the oligonucleotides $GA_{19}$ *(SEQ ID NO:4)* or GA-$_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* were studied. After incubating for 30 minutes at room temperature, the pellet of beads (containing the components which interact with the importins) is separated from the supernatant.

The results are indicated in Figure 10. It appears that the oligonucleotide $GA_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* is combined with the glutathione beads, whereas the

oligonucleotide $GA_{19}$ remains in the supernatant.

This clearly shows the capacity of the oligonucleotide GA-$_{19}$-NLS *(SEQ ID NO:4 and SEQ ID NO:20)* to interact with the α-importin. The peptide part of the oligonucleotide-peptide chimeras is therefore indeed recognized by its receptor, and fulfils, here again, its role as targeting signal.

Page 61, first and second paragraphs:

**Example 9**

This example illustrates the possibility of coupling the oligonucleotide pim-SH *(SEQ ID NO:15)* to the peptide maleimide-NLS *(SEQ ID NO:20)*.

The oligonucleotide pim-SH, of sequence 5'-GGGGAGGGGGAGG-3' (SEQ ID No. 15), with a thiol group at the 5' end, was coupled to the peptide maleimide-NLS *(SEQ ID NO:20)* which possesses a maleimide group at its N-terminal end under the same conditions as for the oligonucleotide $GA_{19}$-SH *(SEQ ID NO:4)* (see Example 5).

Page 61, fourth paragraph:

**Example 10**

This example illustrates the possibility of forming triple helices between the plasmid pXL2997 and the pim-NLS *(SEQ ID NO:15 and SEQ ID NO:20)* chimera in the absence of alkylating agent.

A kinetics of formation of triple helices between the plasmid pXL2997 and the pim-NLS chimera *(SEQ ID NO:15 and SEQ ID NO:20)* (formation obtained as described in Example 8), was carried out and studied according to the technique described in the "Materials and Methods" part, under the section "Formation of triple helices with the

IN THE CLAIMS:

Please amend Claim 23 as follows:

23. (Amended) Vector for transferring nucleic acids according to claim 21, characterized in that the targeting signal is an intracellular targeting signal, such as a nuclear homing sequence (NLS *(SEQ ID NO:20)*).